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Lifitegrast, a Novel Integrin Antagonist for Treatment of Dry Eye Disease

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ABSTRACT The etiology of dry eye disease (DED) is complex and not yet fully understood, but the disease is now recognized as being associated with ocular surface inflammation. The latest advances in the understanding of the pathophysiology of DED have directed the focus of recent drug development to target the inflammatory pathways involved in the disease. Lifitegrast is a novel small molecule integrin antagonist that inhibits T cell-mediated inflammation by blocking the binding of two important cell surface proteins (lymphocyte function-associated antigen 1 and intercellular adhesion molecule 1), thus lessening overall inflammatory responses. This review highlights the role of T cells and integrins in the inflammatory process involved in the pathophysiology of DED and outlines the scientific rationale for the role of lifitegrast. In addition, the preclinical development, pharmacological properties, clinical efficacy, and safety of lifitegrast are described.

KEY WORDS dry eye disease, ICAM-1, inflammation, integrin antagonist, LFA-1, lifitegrast

I. INTRODUCTION

Dry eye disease (**DED**; also referred to as keratoconjunctivitis sicca or dry eye syndrome) is a multifactorial disorder, characterized by either decreased tear production or increased tear film evaporation,[1] which affects both the ocular surface and the lacrimal gland. Although its pathogenesis is not yet fully elucidated, DED is now recognized as a disease associated with ocular surface inflammation.[1] Indeed, the infiltration of T cells in the lacrimal functional unit, including the conjunctiva and lacrimal glands, is known to result in chronic inflammation.

The role of T cells is pivotal in the development of cell-mediated immune responses. More specifically, CD4 positive (+) T helper ($\mathbf{T_H}$) 1 and T_H17 T cells have been identified as mediators of ocular surface inflammation in DED.[2] Recruitment and activation of these T cells at the ocular surface lead to the release of effector cytokines and contribute to the ocular tissue damage seen in patients with DED. In fact, proinflammatory cytokines have been detected in the tear film of patients with DED.[3,4] Therefore, it is hoped that therapies targeting T cells will provide a more efficient means to treat DED.

Currently available treatments include immunomodulators and immunosuppressive agents (e.g., ophthalmic cyclosporine [Restasis®][5] and ophthalmic corticosteroids).[1] Ophthalmic cyclosporine is presently the only approved prescription therapy for use in patients with DED in the United States and Canada. Despite the progress made in recent years in the understanding of the pathophysiology of DED, there is at present no single on- or off-label medication that displays all the following characteristics and benefits of an ideal DED agent: 1) exhibits good tolerability and long-term safety, 2) has a rapid onset of action, 3) targets key steps of the inflammation cycle, and, most importantly, 4) treats both signs and symptoms of DED. Thus, there is an unmet need for new and effective DED therapies, and the recent focus of drug development has been to find novel compounds targeting inflammation.

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OUTLINE

Abbreviations

+	Positive
APC	Antigen-presenting cell
CYP450	Cytochrome P450
DED	Dry eye disease
IC^{50}	Half maximal inhibitory concentration
ICAM-1	Intercellular adhesion molecule 1
ICSS	Inferior corneal staining score
IFN	Interferon
IL-1	Interleukin 1
IS	Immunological synapse
LFA-1	Lymphocyte function-associated antigen 1
MMP	Matrix metalloproteinase
PK	Pharmacokinetic
SD	Standard deviation
T_H	T helper cell
TNF	Tumor necrosis factor
Treg	Regulatory T cell

Lifitegrast is a novel small molecule integrin antagonist that inhibits a specific T cell-mediated inflammatory pathway involved in the pathogenesis of DED. Based on the current understanding of its mechanism of action, lifitegrast blocks the recruitment and activation of T cells to the ocular surface, thus lessening overall inflammatory responses. If approved, lifitegrast has the potential to be the first treatment indicated to treat both signs and symptoms of DED.

Herein, we review the role of T cells and integrins in the inflammatory process involved in the pathophysiology of DED and outline the scientific rationale for the role of lifitegrast. In addition, the preclinical development, pharmacological properties, clinical efficacy, and safety of lifitegrast are described.

II. ROLE OF T CELLS, INTEGRINS, AND ADHESION LIGANDS IN THE INFLAMMATORY PROCESS AND DRY EYE DISEASE

A. Immunology of DED

The pathology of DED is not yet fully understood, but there is growing evidence that T cell-mediated inflammation plays a central role in the disease.[6,7] The role of T cells in DED involves the following 6 steps: 1) uptake and processing of antigens from the ocular tissue by antigen-presenting cells (**APC**) on the ocular surface, 2) priming of T cells by APCs in the lymphoid compartment, 3) migration of T cells through the blood vessels, 4) recruitment of T cells to the conjunctival stroma, 5) activation of T cells, and 6) retention of T cells into inflamed tissues, as illustrated in Figure 1. Specifically, when desiccating environmental stress is applied to the ocular surface, it induces tear hyperosmolarity and the release of proinflammatory cytokines (e.g., interleukin [**IL**]-1 and tumor necrosis factor [**TNF**]-α) via activated kinases.[8] This proinflammatory milieu promotes the activation and maturation of APCs. The migration of mature APCs to lymph nodes in turn triggers the generation of autoreactive CD4+ T cells[6] that journey to the ocular surface, where additional cytokines are produced, thus causing further damage to the corneal epithelium and conjunctival cells (Figure 1).

Understanding the mechanisms involved in the onset and progression of DED is key to the successful development of effective therapeutic interventions. A number of investigational studies and animal models of DED have helped identify and quantify the T cell subtypes and biomarkers (e.g., cytokines, chemokines, and ILs) of ocular inflammation that are implicated in DED. CD4+ T cells, which are found in ocular surface tissues of patients with DED, are the primary infiltrating cells involved in DED.[2,9,10] CD4+ T cells can differentiate via divergent pathways into 4 distinct subsets of T cells, namely T_H1, T_H2, T_H17, and regulatory T cells (**Treg**), depending on which stimuli are driving the onset of inflammation.[11]

Recent human and experimental murine dry eye studies showed that a T_H1- and T_H17-mediated immune response is induced in the lymphoid compartment upon engagement with mature APCs,[4,9,12] as depicted in Figure 1. T_H1 and T_H17 cells subsequently migrate to the ocular surface, where they secrete additional markers of inflammation, in particular interferon (**IFN**)-γ and IL-17, respectively.[3,4] These cytokines in turn promote the production and release of various proinflammatory mediators (including cytokines, chemokines, and matrix metalloproteinases [**MMPs**]) by the conjunctival and corneal epithelium, thus creating a self-perpetuating cycle of inflammation. Relative contributions of T_H1 and T_H17 cells to the pathogenesis of DED are not fully understood, but evidence suggests that IFN-γ causes conjunctival goblet cell loss and apoptosis of the ocular surface epithelium,[3] while IL-17 stimulates the production of MMPs that cause breakdown of the corneal epithelial barrier.[4] When damaged, the corneal epithelium allows greater access of pathogens and inflammatory mediators to the corneal epithelium and stroma (Figure 1), events that may lead to decreased visual function for patients with DED.[12]

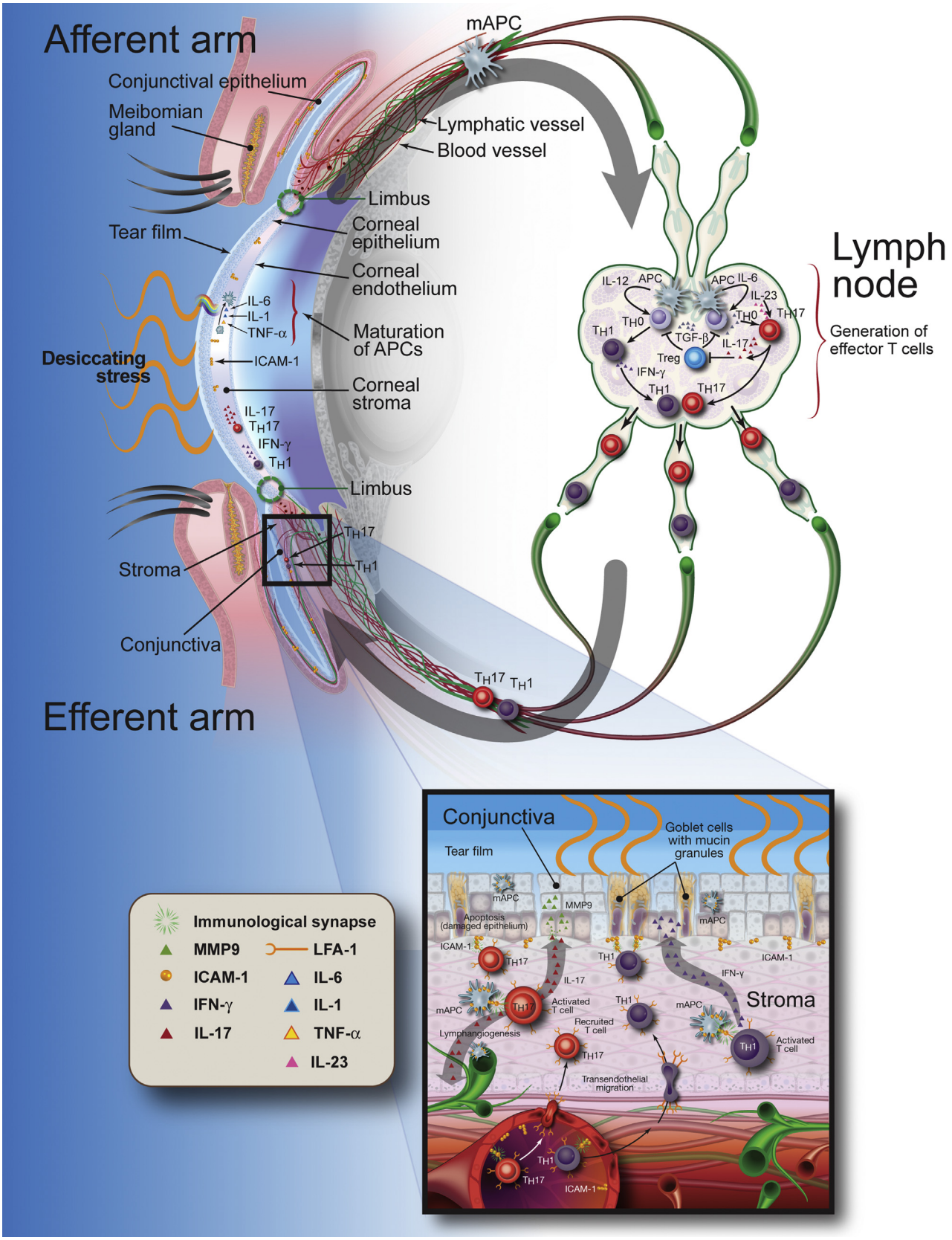

Figure 1. The dry eye immunoinflammatory pathway. APC = antigen-presenting cell; ICAM-1 = intercellular adhesion molecule 1; IFN = interferon; IL = interleukin; LFA-1 = lymphocyte function-associated antigen 1; mAPC = mature antigen-presenting cell; MMP = matrix metalloproteinase; T_H = T helper cell; TNF = tumor necrosis factor; Treg = regulatory T cell.

Taken together, these findings support the idea that inhibiting T cell recruitment and activation by APCs during the development of the inflammatory response in DED should result in decreased levels of pathogenic mediators and less inflammation on the ocular surface.

B. Integrin Signaling in the Immunoinflammatory Pathway

Integrins are cell surface receptors involved in the integration between extracellular and intracellular signals in many biological processes, including cytoskeletal organization and cell adhesion, migration, proliferation, differentiation, and survival.[13] During an immune response, integrins mediate 1) cell adhesion to the extracellular matrix, and 2) cell-cell interactions (e.g., T cell activation), which are central to the pathology of many inflammatory diseases, including DED (Figure 1).

Naïve and memory T cells circulate freely in blood vessels, monitoring for foreign antigens. Integrins are specific heterodimeric receptors used by T cells to routinely migrate in and out of lymph nodes when unchallenged, or into other tissues following activation by an inflammatory signal.[14] At the beginning of an immune response, T cells need to be able to access the site of inflammation by crossing the vascular endothelium of blood vessels. This process is enabled by a specific integrin expressed on T cells and termed lymphocyte function-associated antigen 1 (**LFA-1**), $\alpha_L\beta_2$, or CD11a/CD18, through binding to its native ligand, intercellular adhesion molecule 1 (**ICAM-1**). The expression of LFA-1 is restricted to leukocytes, and LFA-1 is 1 of 12 integrins (out of the 24 known[15]) used by T cells to direct their movement and function.[14]

ICAM-1, an adhesion protein expressed on a variety of cells including APCs and endothelial cells, was first proven to be a ligand for LFA-1 in 1987 by Marlin and Springer.[16] This discovery, together with additional studies, established the understanding of the LFA-1/ICAM-1 pair as a key adhesion pathway in T cell-mediated inflammation.[17,18] Specifically, the interaction of LFA-1 with ICAM-1 is important not only for T cell adhesion to endothelial cells before transendothelial migration to inflamed tissues, but also for T cell interaction with APCs. At the site of inflammation, T cells come into contact with APCs. Upon antigen presentation and recognition, key receptors at the cell-cell interface reorganize to enable the formation of an immunological synapse (**IS**), which stabilizes once ICAM-1, expressed on APCs, is bound to LFA-1.[19,20] The mature IS in turn helps sustain the otherwise transient interaction between the T cell and the APC, which facilitates the propagation of downstream proinflammatory factors, from the T cells themselves and from other bystander cells (Figure 1). In the ocular surface, T cells and corneal epithelial cells produce these signals (e.g., IFN-γ, IL-17, TNF-α, IL-1) accordingly.

TNF-α and IL-1 are known to amplify the inflammatory response by inducing the expression of ICAM-1 on epithelial cells in patients with DED.[21,22] LFA-1 also is upregulated in the conjunctiva of patients with DED.[23] The presence of excess ICAM-1 acts as an activating signal for patrolling T cells in the conjunctival and corneal tissues. It drives the recruitment of additional T cells to the ocular surface through increased LFA-1 expression, thus contributing to the perpetuation of inflammation. Based on the current understanding of DED, blocking the LFA-1/ICAM-1 interaction could be a viable strategy for the prevention and treatment of ocular surface inflammation.

Targeting integrin signaling has been shown to be a valid drug discovery strategy and has allowed the development of drugs with potent anti-inflammatory activities in various autoimmune/inflammatory diseases.[13] For example, efalizumab (Raptiva®),[24] a recombinant humanized monoclonal immunoglobulin G1 antibody against the α subunit of LFA-1 (anti-CD11a), was one of the first integrin antagonists to be marketed for the treatment of moderate to severe psoriasis.[15] It was designed to bind to LFA-1 and block its function in T cell activation in lymph nodes, T cell adhesion and extravasation to inflamed skin, and T cell reactivation in the skin by APCs.[25] Natalizumab (Tysabri®)[26] is another approved drug (for the treatment of relapsing forms of multiple sclerosis) that targets an integrin pathway, specifically the α4-integrin subunit.[27] Targeting integrin signaling systematically can increase the risk of certain rare infections, a side effect that would not be anticipated in a topical medication that reaches systemic circulation at extremely low levels and then is rapidly excreted. Additionally, because lifitegrast is a small molecule antagonist to a specific amino acid sequence of ICAM-1 and not an antibody, it is expected that associated side effects will be minimal.

Preclinical studies in various ocular diseases have shown that inhibiting the interaction between integrins and their ligands, particularly LFA-1 and ICAM-1, holds promise as a therapeutic approach. In a mouse model of induced allergic conjunctivitis, it was established that the greatest inhibition of cellular infiltration in the conjunctiva was achieved with the treatment combination of anti-LFA-1 and anti ICAM-1 monoclonal antibodies,[28] compared with monotherapy with either antibody. In murine endotoxin-induced uveitis, a model for acute inflammation, Becker et al observed a reduced number of infiltrating leukocytes in animals receiving neutralizing antibodies for either LFA-1 or ICAM-1.[29]

These preclinical studies constitute proof-of-concept evidence for targeting integrin signaling in order to reduce ocular surface inflammation. At the time these discoveries were made, it became apparent to experts in the field that if a small molecule that blocked the interaction between LFA-1 and ICAM-1 could be developed, it had the potential to translate into clinical use.

III. DEVELOPMENT OF LIFITEGRAST, AN INTEGRIN ANTAGONIST, AS A TREATMENT FOR DRY EYE DISEASE

Lifitegrast is a novel small molecule integrin antagonist that blocks the binding of ICAM-1 to LFA-1, thus interrupting the T cell-mediated inflammatory cycle.

A. Discovery and Development of Lifitegrast

Protein-protein interactions are central to a majority of biological processes, and are challenging targets to tackle with small molecule inhibitors.[30] These interfaces are large, complex, and difficult to disrupt because of flat surfaces or less-defined binding sites.

In 2002, Gadek et al described the identification of a new series of ICAM-1 mimics and LFA-1 antagonists.[31] It was hypothesized that ICAM-1 could act as a drug discovery lead in the generation of small molecule therapeutics, and the authors succeeded in transferring the binding epitope of ICAM-1 to a small molecule framework. By examining a whole host of molecules through combinatorial chemistry and structure-activity relationship, Gadek et al demonstrated that a molecule coded as Compound 4 (Figure 2) directly inhibited the association of LFA-1 with ICAM-1 by binding to a high-affinity site on LFA-1 (I domain of the α_L subunit).

Between 2010 and 2012, Zhong et al reported the discovery and development of a potent tetrahydroisoquinoline class of LFA-1/ICAM-1 antagonists,[32-34] from which lifitegrast[35] (Compound 1g in Zhong et al[32]; Figure 2) was identified as a promising drug candidate. The central tetrahydroisoquinoline moiety was designed to retain potency of binding affinity to LFA-1.

B. Mechanism of Action of Lifitegrast at the Molecular and Cellular Levels

Based on earlier work on putative ICAM-1 mimics and LFA-1 antagonists (including Compound 4) by Gadek et al[31] and pre-discovery and development of lifitegrast, it has been hypothesized that these molecules bind directly to the ICAM-1 binding site on the I domain of the LFA-1 α_L subunit and act as direct competitive antagonists to block ICAM-1 binding.[36] Alternative attempts to determine the mechanism of inhibition of these compounds (including Compound 4) via surface plasmon resonance experiments suggested that these molecules might not be ligand mimetics of ICAM-1, but that they instead bind to the I-like domain of the LFA-1 β_2 subunit in an allosteric fashion.[37] The mechanism of action of lifitegrast (and other putative ICAM-1 mimics and LFA-1 antagonists) was still under debate until recently. At international congresses in 2013 and 2014, Semba et al reported additional findings on lifitegrast itself, supporting the compound as a direct competitive antagonist of the binding of ICAM-1 to LFA-1 (personal communication, July 2015). In a live-cell experiment[38] created to mimic the binding of LFA-1 to ICAM-1, it was found that lifitegrast inhibited the formation and activation of the IS by affecting LFA-1/ICAM-1 adhesion and by outcompeting ICAM-1 binding to LFA-1 in a dose-dependent fashion. These results confirmed earlier in vitro work demonstrating the ability of lifitegrast to inhibit the attachment of Jurkat T cells to ICAM-1.[39]

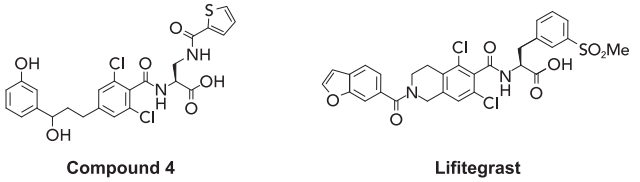

Figure 2. Molecular structures of Compound 4[31] and lifitegrast.[32]

Lifitegrast inhibits the LFA-1/ICAM-1 interaction and as a result should block the subsequent cycle of T cell-mediated inflammation (Figure 3). Lifitegrast's downstream effect on cytokines has been reported in multiple preclinical studies. The drug has been shown to reduce corneal inflammation in mice by inhibiting neutrophil recruitment to the corneal stroma,[40] and to inhibit cytokine release from activated lymphocytes in vitro.[39] Specifically, the inhibitory effect of lifitegrast was significant at 1 μM for IFN-γ, IL-1β, IL-10, and macrophage inflammatory protein 1α, cytokines and chemokines whose presence in tears correlates with the clinical severity of DED.[41] In the phase I clinical study,[42] Semba et al showed that tear concentrations of lifitegrast in healthy volunteers reached, and in some instances exceeded, the target ocular therapeutic level of >1 μM. Additionally, administration of lifitegrast was found to be efficacious in 12 dogs of various breeds, all prone to develop spontaneous keratoconjunctivitis sicca.[39] This body of preclinical evidence confirmed potent dose-dependent inhibition of lifitegrast on the T cell activation, T cell recruitment, and cytokine release steps in the inflammatory process (Figure 3), thus suggesting that treatment with lifitegrast should decrease the inflammatory response and reduce levels of proinflammatory mediators in patients with DED.

C. Lifitegrast: An Ophthalmic Agent for Treatment of Dry Eye Disease

Topical administration is a minimally invasive therapy for patients and has the advantage of increasing the selectivity of a drug for its intended target. Nevertheless, delivering a drug to a specific site of action in the eye is still a challenge for scientists. Lifitegrast was rationally designed and developed to be topically administered as an ophthalmic solution for treating DED. The compound was thus engineered to have a favorable pharmacokinetic (**PK**) profile in the eye, with the following properties:

1) *Strong inhibition of T cell adhesion to ICAM-1 expressing surfaces.* Zhong et al demonstrated that lifitegrast was potent in T cell adhesion assays, including the HUT 78 T cell adhesion assay (half maximal inhibitory concentration [$\mathbf{IC_{50}}$] = 9 nM).[32] Murphy et al showed that lifitegrast strongly inhibited Jurkat T cell attachment to ICAM-1 (IC_{50} = 2.98 nM),[39] thus confirming that lifitegrast inhibits the recruitment of T cells.
2) *High solubility in aqueous media.*[32] Together with drug permeability, solubility is one of the important parameters that helps achieve desired drug concentrations within targeted ocular tissues. Several techniques exist to enhance solubility of a drug compound in aqueous media, including chemical

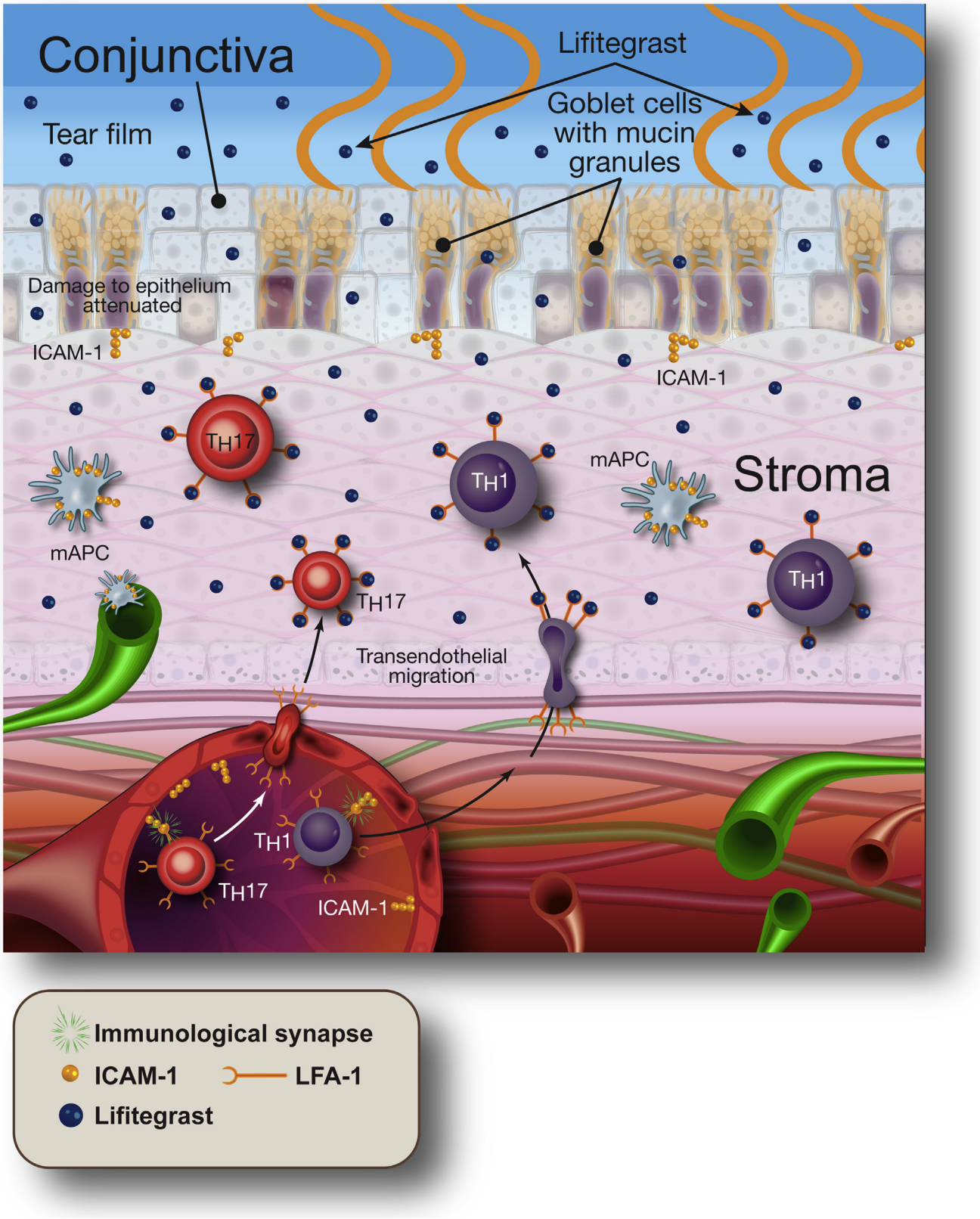

Figure 3. Mechanism of action (MOA) of lifitegrast at the cellular level. ICAM-1 = intercellular adhesion molecule 1; LFA-1 = lymphocyte function-associated antigen 1; mAPC = mature antigen-presenting cell; T_H = T helper cell. Disclaimer: this figure illustrates the current understanding of the MOA of lifitegrast based on completed preclinical and clinical studies. Additional studies in the posterior ocular tissues and vascular system are required to further elucidate the MOA of lifitegrast.

modification by formation of a salt.[43] Lifitegrast is formulated as a sodium salt, which allows for concentrations of ≤100 mg/ml (10%) to be isotonic with human tears at ~300 mOsmol/l. Lifitegrast dosing strengths of ≤50 mg/ml (5.0%) have been used in animal and human studies to maintain the ophthalmic solution at physiological pH.[32] The lifitegrast formulation currently under development for the treatment of DED is a preservative-free 5.0% solution and as such, the product is provided in single-unit dose vials. Lifitegrast's formulation is preservative free in order to minimize aggravation of dry eye that can be caused by such additives.[44]

3) *Rapid absorption into ocular tissues.* Animal models have shown that greater rates of drug penetration and delivery across barriers can be achieved as a result of lifitegrast's high intrinsic solubility and good permeability. The ocular PK of lifitegrast was determined by radiolabeled experiments in rats[45] and dogs.[39] Therapeutic levels of the drug were observed in all ocular tissues, specifically in the bulbar conjunctiva, palpebral conjunctiva, cornea, aqueous humor, vitreous humor, and sclera, 30 min after a single topical ocular administration of ^{14}C-lifitegrast.[45] Ocular penetration also was investigated in dogs, and this has confirmed previous findings.[39] Concentrations of radioactivity were determined to be the highest in the anterior tissues (bulbar conjunctiva, palpebral conjunctiva, and cornea) 30 min post topical dosing. In the human diseased eye, the corneal epithelium and stroma act as barriers between intraocular tissues and the vascular system, thus limiting the permeation of topically administered ophthalmic drugs.[46] In animals, drug levels in ocular tissues can be determined directly through harvesting of the eyes, unlike in humans. Serum plasma concentrations of lifitegrast, determined from patient blood samples, are an indirect measure of the drug's ability to penetrate ocular tissues. Indeed, once a drug accesses posterior ocular tissues, which are highly vascularized, it is subjected to vascular absorption and clearance into the systemic circulation. Peak plasma concentrations of lifitegrast in subjects receiving a single drop of the 5.0% formulation in the phase I clinical trial were detected within 5 min of topical delivery in the eye,[42] thus confirming the rapid absorption of lifitegrast into human ocular tissues.
4) *Rapid clearance from the systemic circulation.* Rat intravenous PK experiments showed a short half-life (0.78 h), high clearance (139.2 ml/min/kg) and low systemic exposure (area under the concentration curve = 705 h*ng/kg) for lifitegrast.[32] This was confirmed in the phase I study in healthy subjects, which established that low plasma levels of lifitegrast were cleared within 1-4 hours of dosing.[42] Additionally, lifitegrast was shown to have good metabolic stability in vitro in both human and rat liver microsomes (71% and >95%, respectively, after 30 min incubation), which contain various drug-metabolizing enzymes including cytochrome P450 (**CYP450**) enzymes.[32] CYP450 enzymes are primarily found in the liver, but they are known to be present in corneal tissues and to participate in drug detoxification.[47,48]
5) *Good safety profile in vitro and in vivo.*[32] The compound was shown to be negative in the Ames test, an assay used to determine whether a chemical can cause mutations in the DNA of the test species (in this instance, bacteria strains). Lifitegrast had low potency in the CYP450 inhibition assay (CYP3A4 [one of the major isoforms], IC_{50} >20 μM; CYP2C9, IC_{50} = 3.0 μM), which tests whether a chemical can affect the activity of CYPs and thus potentially alter drug metabolism in patients,[49] thereby causing therapeutic inefficacy or unanticipated adverse reactions. Additionally, lifitegrast exhibited low potency in the human *ether-à-go-go*-related gene assay (patch clamp, IC_{50} >20 μM), which tests whether a chemical can cause torsades de pointes, thus predisposing a patient to sudden cardiac death. The phase I clinical study in normal healthy adults[42] confirmed that lifitegrast was well tolerated when administered in single and multiple ascending doses. Specifically, subjects did not experience any clinically meaningful changes in their health assessments (vital signs, electrocardiogram, and complete ophthalmologic exam).

In summary, lifitegrast is optimized for ocular use, with an excellent PK profile and a very low systemic exposure. Hence, it is expected to work effectively in the human eye without systemic side effects.

Lifitegrast is currently in late phase III development. The lifitegrast clinical development program is the largest of its kind; it began in 2008 and has enrolled >1,800 patients with DED (placebo and lifitegrast groups). Four clinical studies (3 efficacy and safety studies and 1 long-term exposure safety study) have been completed to date, with further research in progress. Evidence of the efficacy and safety of lifitegrast in patients with DED has been observed in the following clinical studies, which were carried out exclusively in the United States.

In a phase II clinical study (ClinicalTrials.gov identifier, NCT00926185),[50] the group of subjects treated with lifitegrast ophthalmic solution 5.0% did not show a statistically significant difference from the placebo group for the single primary efficacy endpoint (sign), mean inferior corneal staining score (**ICSS**) at day 84 (last visit, week 12). A prespecified secondary sign endpoint, mean (standard deviation, **SD**) change in ICSS from baseline to day 84 (from week 0 to week 12), showed a significant response for the lifitegrast ophthalmic solution 5.0% group compared with placebo (0.05 [0.773] vs 0.40 [0.802], P=.021). Significant improvements in a prespecified secondary symptom endpoint (change on the visual-related function subscale

of a symptom scale) also were noted from baseline to day 84 in the lifitegrast group compared with placebo (−0.30 [0.934] vs 0.07 [0.929], P=.039).[50]

Following the promising findings in the phase II study, the OPUS-1 phase III clinical study (ClinicalTrials.gov identifier, NCT01421498)[51] was conducted between 2011 and 2012, with coprimary objective (sign) and subjective (symptom) efficacy endpoints. Analysis of study results showed that the mean (SD) change from baseline to day 84 in ICSS was greater in the lifitegrast ophthalmic solution 5.0% group compared with placebo (−0.07 [0.868] vs 0.17 [0.819], P<.001). The symptom coprimary endpoint (change on the visual-related function subscale) was not met in this study. However, improvements were noted at day 84 in ocular discomfort in the lifitegrast group compared with placebo (1.10 [1.153] vs 1.31 [1.182], P=.027) and eye dryness in the lifitegrast group compared with placebo (25.00 [28.870] vs 30.39 [30.773], P=.029).[51]

The OPUS-2 phase III clinical study (ClinicalTrials.gov identifier, NCT01743729)[52] was conducted between 2012 and 2013, with coprimary sign and symptom efficacy endpoints. Study results showed that subjects treated with lifitegrast ophthalmic solution 5.0% experienced greater improvement in eye dryness score (mean [SD] change from baseline to day 84) than subjects treated with placebo (−35.30 [28.400] vs −22.75 [28.600], P<.001). Additionally, nominally significant improvements were noted in the secondary symptom endpoints ocular discomfort in the lifitegrast group compared with placebo (−0.91 [1.280] vs −0.57 [1.354], nominal P<.001), and eye discomfort in the lifitegrast group compared with placebo (−26.46 [31.328] vs −16.73 [31.207], nominal P<.001). The sign coprimary endpoint (mean change from baseline to day 84 in ICSS) was not met in this study.[52] In the phase II, OPUS-1 and OPUS-2 studies, lifitegrast was generally well tolerated and there were no serious ocular treatment-emergent adverse events.

The SONATA long-term safety study (ClinicalTrials.gov identifier, NCT01636206) of lifitegrast ophthalmic solution 5.0% is completed. Results from this study were presented at congresses in 2015 and provided further evidence of the safety of lifitegrast. Full results will be published separately. The OPUS-3 phase III clinical study (ClinicalTrials.gov identifier, NCT02284516) is completed. Results will be published separately.

IV. CONCLUSION

Integrin inhibitors have been found to have potent anti-inflammatory effects in several autoimmune/inflammatory diseases. Targeting specific inflammation steps, including integrins and cytokines, is a promising avenue for the development of new and effective therapeutic interventions in DED. Lifitegrast is a novel integrin antagonist specifically developed to target the LFA-1 (an integrin) and ICAM-1 (an intercellular adhesion molecule) interaction. Lifitegrast inhibits T cell recruitment, T cell activation, and subsequent cytokine release, thereby targeting a specific inflammatory pathway involved in the pathogenesis of DED.

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REFERENCES

1. AAO Cornea/External Disease PPP Panel. *Preferred Practice Pattern guidelines. Dry eye syndrome*. San Francisco, CA, American Academy of Ophthalmology. Available at: http://www.aao.org/preferred-practice-pattern/dry-eye-syndrome-ppp-2013; 2013. Accessed 6 October 2015
2. Stern ME, Schaumburg CS, Pflugfelder SC. Dry eye as a mucosal autoimmune disease. *Int Rev Immunol* 2013;32:19-41
3. De Paiva CS, Villarreal AL, Corrales RM, et al. Dry eye−induced conjunctival epithelial squamous metaplasia is modulated by interferon-γ. *Invest Ophthalmol Vis Sci* 2007;48:2553-60
4. De Paiva CS, Chotikavanich S, Pangelinan SB, et al. IL-17 disrupts corneal barrier following desiccating stress. *Mucosal Immunol* 2009;2:243-53
5. *Restasis® (cyclosporine ophthalmic emulsion) 0.05% [prescribing information]*. Irvine, CA, Allergan Inc. Available at: http://www.allergan.com/assets/pdf/restasis_pi.pdf; 2014. Accessed 6 October 2015
6. Schaumburg CS, Siemasko KF, De Paiva CS, et al. Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. *J Immunol* 2011;187:3653-62
7. Stevenson W, Chauhan SK, Dana R. Dry eye disease: an immune-mediated ocular surface disorder. *Arch Ophthalmol* 2012;130:90-100
8. Li DQ, Luo L, Chen Z, et al. JNK and ERK MAP kinases mediate induction of IL-1β, TNF-α and IL-8 following hyperosmolar stress in human limbal epithelial cells. *Exp Eye Res* 2006;82:588-96
9. El Annan J, Chauhan SK, Ecoiffier T, et al. Characterization of effector T cells in dry eye disease. *Invest Ophthalmol Vis Sci* 2009;50:3802-7
10. Stern ME, Siemasko KF, Gao J, et al. Evaluation of ocular surface inflammation in the presence of dry eye and allergic conjunctival disease. *Ocul Surf* 2005;3(Suppl 4):S161-4
11. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* 2008;112:1557-69
12. Chauhan SK, Dana R. Role of Th17 cells in the immunopathogenesis of dry eye disease. *Mucosal Immunol* 2009;2:375-6
13. Millard M, Odde S, Neamati N. Integrin targeted therapeutics. *Theranostics* 2011;1:154-88
14. Hogg N, Laschinger M, Giles K, McDowall A. T-cell integrins: more than just sticking points. *J Cell Sci* 2003;116:4695-705
15. Goodman SL, Picard M. Integrins as therapeutic targets. *Trends Pharmacol Sci* 2012;33:405-12
16. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell* 1987;51:813-9
17. Denucci CC, Mitchell JS, Shimizu Y. Integrin function in T-cell homing to lymphoid and nonlymphoid sites: getting there and staying there. *Crit Rev Immunol* 2009;29:87-109
18. Kavanaugh AF, Lightfoot E, Lipsky PE, Oppenheimer-Marks N. Role of CD11/CD18 in adhesion and transendothelial migration of T cells. Analysis utilizing CD18-deficient T cell clones. *J Immunol* 1991;146:4149-56
19. Grakoui A, Bromley SK, Sumen C, et al. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999;285:221-7
20. Monks CR, Freiberg BA, Kupfer H, et al. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 1998;395:82-6

21. Pisella PJ, Brignole F, Debbasch C, et al. Flow cytometric analysis of conjunctival epithelium in ocular rosacea and keratoconjunctivitis sicca. *Ophthalmology* 2000;107:1841-9
22. Gao J, Morgan G, Tieu D, et al. ICAM-1 expression predisposes ocular tissues to immune-based inflammation in dry eye patients and Sjögrens syndrome-like MRL/lpr mice. *Exp Eye Res* 2004;78:823-35
23. Stern ME, Gao J, Morgan GA, et al. The role of ICAM-1 as a signal protein for predisposition of ocular surface inflammation. *Adv Exp Med Biol* 2002;506(Pt B):753-9
24. *Raptiva® [package insert]*. South San Francisco, CA, Genentech Inc. Available at: http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM143346.pdf; 2009. Accessed 6 October 2015
25. Boehncke WH. Efalizumab in the treatment of psoriasis. *Biologics* 2007;1:301-9
26. *Tysabri® (natalizumab) [prescribing information]*. Cambridge, MA, Biogen Idec Inc. Available at: http://www.tysabri.com/prescribingInfo; 2015. Accessed 6 October 2015
27. Stuve O, Bennett JL. Pharmacological properties, toxicology and scientific rationale for the use of natalizumab (Tysabri) in inflammatory diseases. *CNS Drug Rev* 2007;13:79-95
28. Whitcup SM, Chan CC, Kozhich AT, Magone MT. Blocking ICAM-1 (CD54) and LFA-1 (CD11a) inhibits experimental allergic conjunctivitis. *Clin Immunol* 1999;93:107-13
29. Becker MD, Garman K, Whitcup SM, et al. Inhibition of leukocyte sticking and infiltration, but not rolling, by antibodies to ICAM-1 and LFA-1 in murine endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci* 2001;42:2563-6
30. Arkin MR, Tang Y, Wells JA. Small-molecule inhibitors of protein-protein interactions: progressing toward the reality. *Chem Biol* 2014;21:1102-14
31. Gadek TR, Burdick DJ, McDowell RS, et al. Generation of an LFA-1 antagonist by the transfer of the ICAM-1 immunoregulatory epitope to a small molecule. *Science* 2002;295:1086-9
32. Zhong M, Gadek TR, Bui M, et al. Discovery and development of potent LFA-1/ICAM-1 antagonist SAR 1118 as an ophthalmic solution for treating dry eye. *ACS Med Chem Lett* 2012;3:203-6
33. Zhong M, Hanan EJ, Shen W, et al. Structure-activity relationship (SAR) of the α-amino acid residue of potent tetrahydroisoquinoline (THIQ)-derived LFA-1/ICAM-1 antagonists. *Bioorg Med Chem Lett* 2011;21:307-10
34. Zhong M, Shen W, Barr KJ, et al. Discovery of tetrahydroisoquinoline (THIQ) derivatives as potent and orally bioavailable LFA-1/ICAM-1 antagonists. *Bioorg Med Chem Lett* 2010;20:5269-73
35. *Shire to acquire Sarcode Bioscience, expands presence in ophthalmology [news release]*. Lexington, MA, Shire. Available at: http://www.shire.com/newsroom/2013/march/shire-to-acquire-sarcode; March 25, 2013. Accessed 6 October 2015
36. Keating SM, Clark KR, Stefanich LD, et al. Competition between intercellular adhesion molecule-1 and a small-molecule antagonist for a common binding site on the αl subunit of lymphocyte function-associated antigen-1. *Protein Sci* 2006;15:290-303
37. Shimaoka M, Salas A, Yang W, et al. Small molecule integrin antagonists that bind to the β_2 subunit I-like domain and activate signals in one direction and block them in the other. *Immunity* 2003;19:391-402
38. Hartman NC, Nye JA, Groves JT. Cluster size regulates protein sorting in the immunological synapse. *Proc Natl Acad Sci U S A* 2009;106:12729-34
39. Murphy CJ, Bentley E, Miller PE, et al. The pharmacologic assessment of a novel lymphocyte function-associated antigen-1 antagonist (SAR 1118) for the treatment of keratoconjunctivitis sicca in dogs. *Invest Ophthalmol Vis Sci* 2011;52:3174-80
40. Sun Y, Zhang R, Gadek TR, et al. Corneal inflammation is inhibited by the LFA-1 antagonist, lifitegrast (SAR 1118). *J Ocul Pharmacol Ther* 2013;29:395-402
41. Lam H, Bleiden L, de Paiva CS, et al. Tear cytokine profiles in dysfunctional tear syndrome. *Am J Ophthalmol* 2009;147:198-205
42. Semba CP, Swearingen D, Smith VL, et al. Safety and pharmacokinetics of a novel lymphocyte function-associated antigen-1 antagonist ophthalmic solution (SAR 1118) in healthy adults. *J Ocul Pharmacol Ther* 2011;27:99-104
43. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. *ISRN Pharm* 2012;2012:195727
44. Fraunfelder FT, Sciubba JJ, Mathers WD. The role of medications in causing dry eye. *J Ophthalmol* 2012;2012:285851
45. Rao VR, Prescott E, Shelke NB, et al. Delivery of SAR 1118 to the retina via ophthalmic drops and its effectiveness in a rat streptozotocin (STZ) model of diabetic retinopathy (DR). *Invest Ophthalmol Vis Sci* 2010;51:5198-204
46. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. *AAPS J* 2010;12:348-60
47. Duvvuri S, Majumdar S, Mitra AK. Role of metabolism in ocular drug delivery. *Curr Drug Metab* 2004;5:507-15
48. Vadlapatla RK, Vadlapudi AD, Pal D, Mitra AK. Role of membrane transporters and metabolizing enzymes in ocular drug delivery. *Curr Drug Metab* 2014;15:680-93
49. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician* 2007;76:391-6
50. Semba CP, Torkildsen GL, Lonsdale JD, et al. A phase 2 randomized, double-masked, placebo-controlled study of a novel integrin antagonist (SAR 1118) for the treatment of dry eye. *Am J Ophthalmol* 2012;153:1050-1060.e1
51. Sheppard JD, Torkildsen GL, Lonsdale JD, et al, OPUS-1 Study Group. Lifitegrast ophthalmic solution 5.0% for treatment of dry eye disease: results of the OPUS-1 phase 3 study. *Ophthalmology* 2014;121:475-83
52. Tauber J, Karpecki P, Latkany R, et al, OPUS-2 Investigators. Lifitegrast ophthalmic solution 5.0% versus placebo for treatment of dry eye disease: results of the randomized phase III OPUS-2 study. *Ophthalmology* 2015;122:2423-31